Protocol of constructing mCherry-SpyTag

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1. Target sequence for Gibson assembly into pET22b(+)

AATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAA**G**AAGGA
GATATA**CAT**ATGGCGCACATCGTTATGGTCGATGCATATAAACCCACCAAATACGGTGGCGGTGGCTCTGGTGGCGG
TGGCTCTATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGAC
CGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCT
CCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGA
GCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCAT
CTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACGATGGGCTGGGA
GGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGG
ACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACA
ACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGG
CCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGCGGGTCTCGAGCACCACCACCACCACCACTGAGATCCG
GCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGG
CCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT

Red: mCherry
GGTGGCGGTGGCTCTGGTGGCGGTGGCTCT: linker
Blue: SpyTag

First

Amplify the mCherry twice, each time with a flanking sequence on the left to include SpyTag. The F2 and R included the restriction site. PCR progression: 98(1:00)-**98(0:10)-58/60(0:30)-72(1:00)**-72(5:00). The bold sequence for 34x.

F1:
CATATAAACCCACCAAATACGGTGGCGGTGGCTCTGGTGGCGGTGGCTCTATGGTGAGCAAGGGCGAGGA

F2:
ATAT*CATATG*GCGCACATCGTTATGGTCGATGCATATAAACCCACCAAATA

R:ATA TCT CGA GAC CCG CCT TGT ACA GCT CGT

Then

PCR to add Gibson sequence. We used Easy Pure Quick Gel Extraction Kit to purify the sequences for Gibson Assembly. The Gibson Assembly protocol should refer to the specific product instruction.

F_Gibson:
TTTGTTTAACTTTAA**G**AAGGAGATATA**CAT**ATGGCGCACATCGTTAT

R: Gibson
ATC TCA GTG GTG GTG GTG GTG GTG CTC GAG ACC CGC CTT GTA CAG CTC GT

PCR protocol	
template	0.5
F	1
R	1
ddH2O	20
Q5(NEB) 2xMix	22.5